



Reagent D

Revision A, September 2023

For the elimination of DNA from dead cells to avoid false-positive PCR results.

Product No. KIT230001

30 mL

Product No. KIT230002

150 mL

Product No. KIT230003

15 mL

Store in the dark at -15 to -25 °C

For food testing purposes

FOR *IN VITRO* USE ONLY



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1. What this Product Does

The Reagent D is designed for the rapid elimination of DNA from dead cells to avoid false-positive PCR results. The reagent contains a light sensitive substance which can only penetrate the cell membranes of dead cells. Exposure to visible light leads to covalent binding of this substance to DNA and prevents the DNA from being amplified via PCR.

1.1 Kit Contents

Product	Content	Storage
KIT230001	3 bottles containing 10 mL reagent each	-15 to -25 °C
KIT230002	3 bottles containing 150 mL reagent each	-15 to -25 °C
KIT230003	15 bottles containing 15 mL reagent each	-15 to -25 °C

1.2 Storage and Stability

Reagent D is guaranteed to be stable through the expiration date on the label when stored at -15 to -25 °C. Avoid freezing and thawing for more than 3 times. Reagent D may lose activity when stored for more than 7 days at 2 - 8 °C.

1.3 Additional Equipment Required

- foodproof® D-Light (Product No. MCH230039)

Alternatively:

- Lamp with a high-power halogen light bulb (approx. 500 W)
- Cooling block for reaction (microcentrifuge) tubes (1.5 mL)

1.4 Applicability Statement

3 x 10 mL (Product No. KIT230001) and 15 x 10 mL (Product No. KIT230002) are recommended when testing for
Enterobacteriaceae in instant milk formula (IMF)
Enterobacteriaceae plus ***Salmonella***
Yeast and Mold in dairy products
Legionella in environmental samples

3 x 5 mL (Product No. KIT230003) is recommended when testing for
Vibrio in food matrices

The reagent might not work with products strongly impeding the penetration of visible light. The reagent can be directly used in combination with Hygiena Diagnostics GmbH's DNA isolation kits.



1.5 Elimination Procedure

In order to avoid cross-contamination use filter tips. Follow all universal safety precautions governing work with bio- and chemical-hazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

Note: Reagent D has a mutagenic potential and the hazardousness has not been thoroughly investigated. Always wear gloves and handle this substance with caution.

Always keep Reagent D cool and protect from light during storage and handling.

Reaction tubes should be transparent and must have a transparent cap or lid. If they are numbered on the cap, only use thin pencils to ensure light transfer through the cap into the tube or open the cap carefully before the exposure step in the protocol of the respective DNA isolation kit.



2. How to Use this Product

2.1 Procedure A: foodproof® Enterobacteriaceae plus Cronobacter Detection Kit in combination with foodproof® StarPrep® One

Step	Action	Volume Time/g Time/Temperature
1	Shake the enrichment culture gently and let settle . Note: For sub-cultivation at small scale (e.g., 2 mL reaction tubes) only mix carefully by stirring with a pipette tip during step 3.	5 – 10 min
2	Pipet Reagent D into an empty 1.5 mL (transparent) reaction tube. Note: Proceed immediately with the following steps of the protocol. Avoid extended exposure to light.	300 µL
3	Transfer the sample (supernatant) into the reaction tube already filled with Reagent D. Mix thoroughly by pipetting up and down. Note: For very cloudy supernatants, a reduction of the sample volume (e.g., 50 µL) might enhance the efficiency.	100 µL
4	Incubate in the dark.	5 min at RT
5	Exposure to a high-power halogen light bulb. Note: Place the reaction tubes approximately 20 cm from the light bulb on ice or in a cooling block to minimize elevated temperature in the samples. The light should pass the cap into the sample.	5 min
6	Centrifuge . Note: If the enrichment cultures are totally clear, centrifugation at 13,000 × g is recommended. Use e.g., latex beads to increase efficiency and yield a visible pellet.	5 min at 8,000 × g
7	Remove supernatant with a pipette immediately after centrifugation, discard, and inactivate appropriately. Note: Take care that the tip of the pipette in the reaction tube is on the opposite side of the pellet.	
8	Add Lysis Buffer . Note: Use a magnetic stirrer (low speed) or shake the bottle with Lysis Buffer gently in a short time interval to avoid sedimentation of ingredients.	200 µL
9	Resuspend pellet by vortexing or by pipetting gently up and down. Note: For optimal DNA isolation efficiency, the pellet has to be completely resuspended.	
10	Incubate suspension in a heating unit.	10 min at 95 – 100 °C
11	Carefully remove reaction tube from the heating unit and allow the tube to sit . As the tube will be hot, use forceps for removal.	1 min at room temperature
12	Mix by vortexing.	2 s
13	Centrifuge . Result: The supernatant now contains the extracted DNA and can be used directly for PCR. Note: Parts of the sediment may inhibit PCR and must not be used for PCR.	2 min at 13,000 × g



2.2 Procedure B: foodproof *Enterobacteriaceae plus Salmonella* LyoKit in combination with foodproof StarPrep Three

Please refer to the manual of the foodproof StarPrep Three Kit (“Product Instructions for *Enterobacteriaceae plus Salmonella*”) on how to apply Reagent D during the extraction process.

2.3 Procedure C: foodproof *Yeast & Mold Quantification* LyoKit in combination with foodproof StarPrep Two

Please refer to the manual of the foodproof StarPrep Two Kit (“Product Instructions for *Yeast and Mold*”) on how to apply Reagent D during the extraction process.

2.4 Procedure D: foodproof *Vibrio Detection* LyoKit in combination with foodproof StarPrep Three

Please refer to the manual of the foodproof StarPrep Three Kit (“Product Instructions for *Vibrio*”) on how to apply Reagent D during the extraction process.

2.5 Procedure E: microproof *Legionella Quantification* LyoKit in combination with foodproof StarPrep Two

Please refer to the manual of the foodproof StarPrep Two Kit (“Product Instructions for *Legionella*”) on how to apply Reagent D during the extraction process.

3. Troubleshooting

Problem	Possible cause	Recommendation
DNA elimination not effective.	Inappropriate storage of Reagent D.	Store at -15 to -25 °C in the dark. Avoid freezing and thawing for more than 3 times. Aliquots should be prepared and stored in dark.
	Reagent D inactive.	After thawing, always keep Reagent D refrigerated. Reagent D may lose activity when stored for more than 7 days at 2 - 8 °C.
	Enrichment culture contains substances that reduce the light transmission.	Perform a sub-cultivation (e.g., 1:10 dilution in fresh enrichment broth) or reduce the sample volume.
	Enrichment culture contains substances that inhibit Reagent D.	Prepare a 1:10 dilution in fresh enrichment broth or reduce the sample volume.

4. Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability.” Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

- (1) The product is used according to the guidelines and instructions set forth in the product literature;
- (2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or if the product is contaminated by improper storage or handling;



(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;

(5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

5. Supplementary Information

5.1 Ordering Information

Hygiena Diagnostics GmbH is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

5.2 Trademarks

foodproof is a trademark of Hygiena Diagnostics GmbH.

Other brand or product names are trademarks of their respective holders.

5.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (for details see www.hygiena.com). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

5.4 Reference number

The reference number and original Hygiena Diagnostics GmbH article number: A500 02 20 (L/S)

6. Change Index

Version 1, October 2007:

First version of the package insert.

Version 2, May 2008:

Page 3, Elimination Procedure.

Version 3, April 2016:

Additional products KIT230002 and KIT230003.

Revision A, September 2023:

Rebranding and new layout.

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